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Time Course of Capsaicin-Induced Functional Impairments in Comparison with Changes in Neuronal Substance P Content

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Summary. 1. Changes in the content of substance P (dorsal spinal cord, dorsal roots, dorsal root ganglia, saphenous nerve, skin) and functional changes (neurogenic plasma extravasation, chemosensitivity of the cornea) were measured in the rat from 10 min to 4 days after the s.c. injection of a single dose of 50 mg kg^{-1} capsaicin.

2. The substance P content in dorsal roots, saphenous nerve and hind paw skin progressively declined to about 60–70% of control 4 days after treatment, whereas that of the dorsal root ganglia rose, after an initial decline, to 140% after 1–4 days.

3. After denervation, impairment of neurogenic plasma extravasation could be observed not earlier than after one day, thus being comparable in time course to the depletion of substance P in the skin and saphenous nerve.

4. Neurogenic plasma extravasation and the chemosensitivity of the cornea were greatly diminished already 10 min after systemic capsaicin treatment, i.e. at a time when the substance P content of the peripheral nerve was still unchanged. These early effects of systemic capsaicin treatment are therefore caused by actions other than depletion of substance P.

Key words: Substance P – Capsaicin – Chronic denervation – Neurogenic plasma extravasation – Chemosensitivity

Introduction

Two effects of capsaicin have been observed on substance P containing neurones. First, capsaicin releases substance P from the spinal cord as shown in vitro (Gamse et al. 1979a; Theriault et al. 1979) and in vivo (Yaksh et al. 1980). It appears that capsaicin releases substance P only from sensory substance P neurones (Gamse et al. 1979a; 1981). Capsaicin-induced release of substance P from sympathetic ganglia, presumably from peripheral endings of primary sensory neurones, was also demonstrated (Gamse et al. in press).

The second effect of capsaicin, observed after systemic administration, is a long-lasting reduction of the substance P content which is apparently confined to regions that contain primary sensory neurones (Jessell et al. 1978; Gamse et al. 1980a; Nagy et al. 1980). The reduction of the substance P content by capsaicin seems to be irreversible in the rat if capsaicin is given within 10 days after birth, but reversible if animals are treated later in life (Gamse et al. 1980a). A recent finding showed a drastic (90%) reduction of substance P biosynthesis in isolated sensory ganglia of rats pretreated neonatally with capsaicin (Harmar et al. 1980). Morphologically, neonatal capsaicin treatment causes a selective degeneration of thin unmyelinated sensory fibres (Jancsó et al. 1977; Scadding 1980).

Systemic treatment with capsaicin results also in other biochemical and in functional changes. Opiate binding (Gamse et al. 1979b; Nagy et al. 1980) is reduced in the dorsal spinal cord. The nociceptive threshold to mechanical and chemical stimuli is markedly elevated (Jancsó et al. 1977; Hayes and Tyers 1980). The threshold for noxious heat was found to be only moderately increased (Holzer et al. 1979; Nagy et al. 1980) or unchanged (Hayes and Tyers 1980). Antidromic vasodilatation and neurogenic plasma extravasation, both processes that are dependent on the integrity of sensory fibres and probably mediated by substance P, are nearly abolished after capsaicin pretreatment (Jancsó et al. 1967, 1977; Lembeck and Holzer, 1979; Gamse et al. 1980a). It is still not clear whether there is a relation between the release and depletion of substance P by capsaicin and the observed functional changes seen after capsaicin treatment. Release and depletion of somatostatin from sensory neurons by capsaicin (Gamse et al. 1980b, 1981) has to be considered in addition.

The present investigation was aimed at monitoring the time course of changes in substance P content, neurally evoked plasma extravasation and chemosensitivity of the cornea after the subcutaneous injection of a single dose of capsaicin.

Methods

a) Animals. Sprague-Dawley rats (strain OFA, SD, SPF; 200–300 g) of either sex were used. To avoid excessive pain and motor activity, injections of capsaicin were carried out under light pentobarbital anaesthesia (20 mg kg^{-1} i.p.). Capsaicin was injected in a dose of 50 mg kg^{-1} s.c. in the neck. Control animals received solvent only (10% ethanol, 10% Tween 80 in saline). Experiments or dissections were performed at 10, 30, 100, 300 min and 1, 2, 4 days after the injection.

b) Plasma Extravasation Induced by Mustard Oil. Under pentobarbital anaesthesia (50 mg kg^{-1} i.p.) the trachea was cannulated, and body temperature was measured by a rectal thermometer and kept constant with a warming lamp. Evans Blue (50 mg kg^{-1} i.v.) was injected. After 5 min the dorsal side of one hind paw was painted with 5% (w/w) mustard oil in liquid paraffin three times at 5 min intervals (Gamse et al. 1980a). Paraffin oil alone was applied to the control paw. Fifteen minutes after the first painting the rats were killed by bleeding and the skin of the dorsal side of the hind paw was removed.

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c) Neurogenic Plasma Extravasation. Under pentobarbital anaesthesia both saphenous nerves were carefully exposed, cut in the thigh and the distal ends were placed on bipolar platinum electrodes. The nerves were covered with paraffin oil. Fifteen minutes after the electrodes had been placed, Evans Blue was injected i.v.; 5 min afterwards one nerve was stimulated for 5 min (20 V/2 ms/5 Hz); the other one served as a control. After the end of stimulation, the rats were killed and the skin areas of both sides which are innervated by the saphenous nerve were removed.

d) Extravasated Evans Blue was extracted for colorimetric determination as described by Gamse et al. (1980a). The amount of Evans Blue exceeding that of the control paw was used to quantify neurogenic plasma extravasation induced by mustard oil (see b) or electrical stimulation (see c).

e) Weight of dorsal root ganglia was determined before and after drying at 100° C for 10 h. The protein content of the tissue was measured by the method of Markwell et al. (1978).

f) Blood flow in the hind paw of rats was measured by the outflow from the femoral vein with an electronic drop recorder connected to a Beckman dynograph (Lembeck and Holzer 1979). Systemic blood pressure was recorded from a carotid artery with a Statham pressure transducer and kept constant by infusion of heparinized rat blood into the jugular vein.

g) Chemosensitivity of the cornea following the s.c. injection of capsaicin was measured by the number of protective scratching-wiping movements ("wiping test") in response to a 0.1 mg ml^{-1} capsaicin solution (Szolcsányi and Jancsó-Gábor 1975).

h) Determination of Substance P. Rats were killed by a blow on the neck. The lumbar spinal cord was removed, chilled, and the dorsal roots were dissected. The spinal cord was frozen on dry ice, and cut into thin slices from which the dorsal halves were separated. Finally, dorsal root ganglia, the saphenous nerve and the skin of the dorsal side of the hind paw were removed.

Tissue from central and peripheral nervous system was boiled for 5 min in about ten volumes of 2 M acetic acid, homogenized by sonification and centrifuged. The pellet was washed with 0.5 ml 2 M acetic acid and the combined supernatants were lyophilized. By this method of extraction substance P was recovered quantitatively. The skin was pulverized in liquid nitrogen and extracted with acetone-HCl, according to Chang and Leeman (1970). Substance P was measured with a sensitive and specific radioimmunoassay, as described by Gamse et al. (1979c).

i) Chronic Denervation. Under pentobarbital anaesthesia the sciatic and saphenous nerves were cut in the thigh; control rats were sham operated.

k) Substances Used. Capsaicin (Merck, Darmstadt, FRG), Substance P (Peninsula, San Carlos, CA, USA).

Results

A. Functional Changes

After denervation, a slow time course was found for the decrease of plasma extravasation induced by cutaneous application of mustard oil. Plasma extravasation was still as high as in controls 100 min after denervation, reduced to 50 % after 1 day, and down to 7 % after 2 days (Fig. 1 A).

Plasma extravasation induced by mustard oil was reduced to 25 % already 10 min after the subcutaneous injection of capsaicin; it was even more reduced after 30 min and 5 h, and remained lowered to about 40 % after 1 or 4 days (Fig. 1 B). The pronounced initial reduction may to some extent result from decreased blood flow caused by capsaicin as the outflow from the femoral vein was found to be decreased to about 60 % ($n = 3$) 30 min after the s.c. injection of capsaicin. Plasma extravasation induced by electrical stimulation of the saphenous nerve was decreased to a similar degree and as early as that induced by mustard oil (Fig. 1 C).

Protective wiping movements in response to a 0.1 mg ml^{-1} solution of capsaicin applied on to the eye ("wiping test") were completely absent already 10 and 30 min after systemic capsaicin treatment and remained absent for 4 days, the longest time tested (Fig. 1 D).

B. Changes in Substance P Content

The substance P content in dorsal roots, saphenous nerve and hind paw skin progressively declined to about 60 – 70 % after 4 days (Fig. 2 B, D, E). The apparent slight initial increase of substance P in dorsal roots and hind paw skin was not significant (Fig. 2, B, E). The beginning of the decline is difficult to estimate as the changes within 24 h are minimal. There was no marked change in the substance P level of the dorsal spinal cord (Fig. 2 A). The substance P content of the dorsal root ganglia was apparently though not significantly lowered to 70 % of controls already 10 min after the capsaicin administration and remained decreased for 5 h. Swelling of the ganglia was excluded as a possible cause for these unexpected results, since there was no difference in wet weight (8.4 ± 0.9 mg against 8.0 ± 0.9 mg), in dry weight (3.1 ± 0.4 mg against 2.8 ± 0.2 mg) or in protein content (89.9 ± 3.2 µg/mg wet wt. against 92.3 ± 1.8 µg/mg wet wt.) between ganglia of capsaicin treated and control rats ($n = 4$).

After the initial decrease, the substance P content of dorsal root ganglia was found to be elevated to about 140 % of controls 1, 2 and 4 days after treatment. This finding, first made in a set of 6 rats, was fully reproduced in another set of 6 rats (Fig. 2 C).

Discussion

The denervation experiment shows that the function of the distal nerve stump, as measured by the neurogenic plasma extravasation, disappears slowly. Degenerative processes that set in after nerve section are assumed to deplete the neurogenic factor that mediates plasma extravasation. The time course of impairment of neurogenic plasma extravasation (Fig. 1 A) coincides very well with the time course of depletion of substance P determined by Jessell et al. (1979) in the distal stump of the cut sciatic nerve.

The depletion of substance P after capsaicin treatment was also a slow process. Only one dose of capsaicin was injected subcutaneously in order to monitor the development of the early changes in substance P content and related functional impairments. This dose of 50 mg kg^{-1} was lower than the doses generally used for depletion of substance P in adult rats which is 2 (Gamse et al. 1980a) or even 19 times (Jessell et al. 1978) higher. Therefore, only a moderate depletion of substance P could be expected. Depletion of substance P in the hind paw skin commenced not earlier than 5 h after the s.c. injection of capsaicin (Fig. 2 B, D, E). This slow decline of the content of substance P contrasts to the almost immediate functional impairment after the s.c. injection of capsaicin (Fig. 1 B, C).

The reduced neurogenic plasma extravasation and the abolished chemosensitivity of the cornea (Fig. 1) can, therefore, not result from depletion of substance P but either from tachyphylaxis to capsaicin as observed after repeated administration of capsaicin in vivo (Szolcsányi et al. 1975) and in vitro (Barthó and Szolcsányi 1978; Gamse et al. 1979a) or other biochemical changes. An immediate effect of capsaicin is the release of substance P as shown in vitro and in vivo

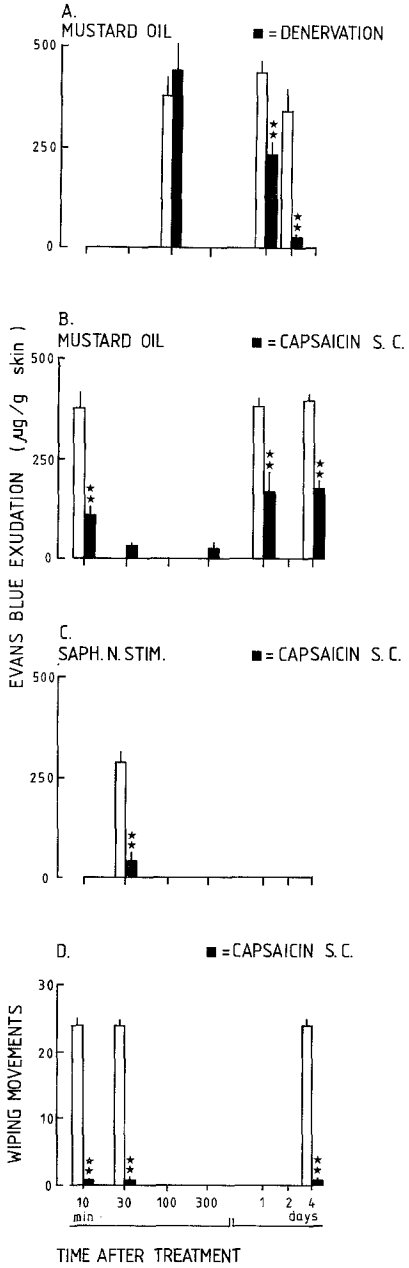

Fig. 1. (**A**) Evans Blue content of the hind paw skin following painting with 5% mustard oil. Effect of denervation of the hind paw. Denervated paw: black columns, control paw: blank columns. (**B, C**) Neurogenic plasma extravasation in the rat hind paw induced by painting with 5% mustard oil (**B**) or antidromic stimulation (20 V, 2 ms, 5 Hz, 5 min) of the cut saphenous nerve (**C**). Effect of treatment with capsaicin (50 mg kg^{-1} s.c.). Capsaicin treated rats: black columns, control rats: blank columns. (**D**) Protective wiping movements in response to capsaicin (0.1 mg ml^{-1}) on the eye; effect of treatment with capsaicin (50 mg kg^{-1} s.c.). Capsaicin treated rats: black columns, control rats: blank columns. Values are means ± SEM; $n = 6$. * $P < 0.05$, ** $P < 0.01$ (Student's paired t-test for the effect of denervation; otherwise two-sample t-test)

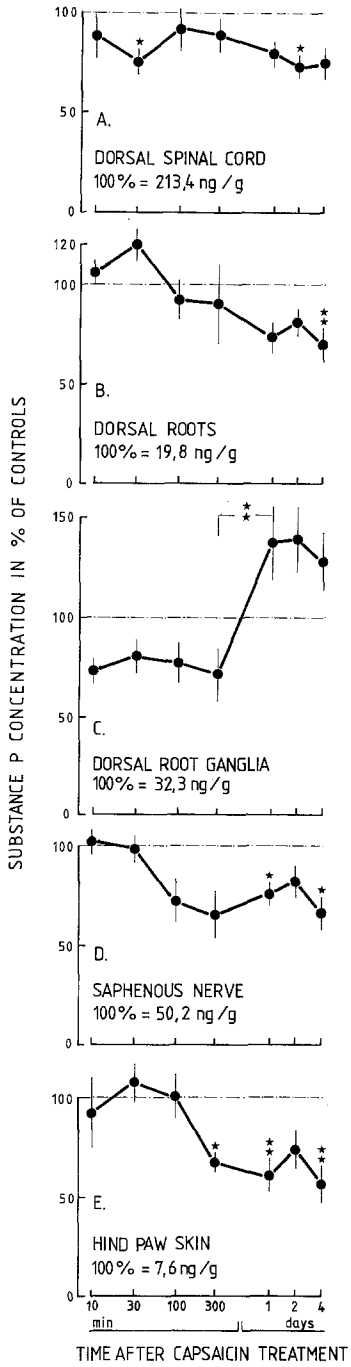

Fig. 2. Effect of a single dose of capsaicin (50 mg kg^{-1} s.c.) on the substance P content of the dorsal spinal cord (**A**), dorsal roots (**B**), dorsal root ganglia (**C**), saphenous nerve (**D**), and hind paw skin (**E**). The percental differences in substance P content of capsaicin treated versus untreated controls are shown. The difference between the substance P content at 300 min and at 1 day in capsaicin treated animals is indicated in part C by a bracket. Values are means ± SEM; $n = 6$. * $P < 0.05$, ** $P < 0.01$ (Student's two-sample t-test).

(Gamse et al. 1979a; Theriault et al. 1979; Yaksh et al. 1980). It cannot be excluded that a prolonged release of substance P by capsaicin in vivo could cause a depletion of substance P. Metabolic changes like inhibition of protein synthesis by capsaicin, which were discussed by Jóo et al. (1969) and Szolcsányi et al. (1975), can hardly explain the fast functional impairments.

The delayed decline of the substance P content in dorsal roots, saphenous nerve and skin after injection of 50 mg kg^{-1} capsaicin is in contrast to the prompt reduction of substance P in dorsal root ganglia (Fig. 2C). The latter finding remains unexplained after exclusion of swelling of the tissue by capsaicin as a possible cause. Whereas the substance P content in dorsal roots, saphenous nerve and hind paw skin

was decreased 1 day after the injection of capsaicin, the substance P content in the dorsal root ganglia was raised to about 140% of the content in control rats after 1–4 days. Such an increase of substance P has also been observed by Gamse (personal communication) after s.c. injection of 50 mg kg^{-1} but not after higher doses of capsaicin.

The fast functional impairments observed after s.c. injection of capsaicin are to be considered in view of the pharmacokinetic characteristics of capsaicin. Capsaicin was shown to rapidly enter the central nervous system of the rat; following an i.v. injection of 2 mg kg^{-1}, the concentration of capsaicin in central nervous system reached 5 times its level in the blood within 3 min (Saria et al. 1981). Capsaicin could be detected in brain and spinal cord already 10 min after s.c. injection of 50 mg kg^{-1}; the concentration of capsaicin in these tissues rose for up to 5 h and declined in the following 12 h to very low values (Saria et al. in press). The fast decrease of neurogenic plasma extravasation and of the chemosensitivity of the cornea thus coincides with the fast entry of capsaicin into nervous tissue upon s.c. injection. The depletion of substance P occurs, however, with a considerable delay and still progresses after the elimination of capsaicin from the body. Also the observed rise of the substance P content in the dorsal root ganglia coincides fairly well with the end of the elimination of capsaicin (Saria et al. in press). It appears that the loss of substance P in the central and peripheral branches of sensory fibres leads to a reactive increase in biosynthesis of substance P in the dorsal root ganglia.

Capsaicin has many actions. Already very small doses render perivascular nociceptors insensitive to bradykinin or acetylcholine (Juan et al. 1980) and polymodal nociceptors in the skin insensitive to chemical and thermal noxious stimuli (Szolcsányi 1977). Capsaicin releases not only substance P and somatostatin from nerves but also prostaglandins from other tissues (Juan et al. 1980); both effects are dependent on the extracellular calcium concentration (Gamse et al. 1979a, 1981; Juan et al. 1980). In view of these results it is tempting to think of biochemical changes occurring within the cell membrane of sensory fibres. It is assumed that these biochemical changes induce, after an initial release of substance P from the nerve endings, a progressive slow depletion of substance P in the nerve fibre.

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